winding. Perhaps the runs of dihydrouracil in chromosomal RNA (Huang and Bonner, 1965) are anchored to a matching set of runs of poly(dA) in the DNA genome *via* interactions at the adenine Hoogsteen site. If the excess functional groups of the other bases are also reactive toward their complimentary counterparts this suggests an interesting mechanism of transcription using the intact but perhaps slightly distorted Watson and Crick duplex as the template.

We have also demonstrated in these experiments, the feasibility and advantages of employing two techniques of a different nature for the study of a very complex set of weak interactions. For such cases, utilization of a single technique can lead to serious errors in the interpretation of data and the value of the associations constants. Since the mixed association of A and U is occasionally used as a standard to test other methods, we suggest that the more accurate values of the association constants presented in this and the preceding paper be employed. In addition, these values also permit an accurate evaluation of the more pertinent enthalpies of the various associative processes by calorimetric methods.

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Studies on the Conformation of Purine Nucleosides and Their 5′-Phosphates†

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ABSTRACT: Circular dichroism of various purine nucleosides and their 5′-monophosphates was measured. From the relative rotational strength and the sign of the Cotton effect following conclusions were reached. (1) Purine nucleosides having bulky substituents in 8 position, such as bromo, 2-hydroxypropyl, and methylmercapto group, have syn conformations. (2) Introduction of a phosphate to the 5′-hydroxyl group of anti-type nucleosides, such as adenosine, 8,2′- and 8,3′-*S*-cycloadenosine, does not change the circular dichroism (CD) curve in the B-band region. (3) Introduction of a phosphate to the 5′-hydroxyl group of syn-type nucleosides such as 8-substituted purine nucleosides caused a drastic change of CD curves over a wide range of wavelength. (4) Both in syn- and anti-type purine nucleosides, introduction of the 5′-phosphate caused a significant change in the Cotton band of 200–220 nm.

Recently, optical rotatory dispersion (ORD) and circular dichroism (CD) of purine and pyrimidine nucleosides were extensively investigated for the elucidation of the conformation of nucleosides, nucleotides, and polynucleotides (Yang and Samejima, 1969; Miles *et al.*, 1969). A theoretical study for the calculation of rotational strength of nucleosides has been reported in order to interpret the effect of substituents to the base (Miles *et al.*, 1967) and the sugar moiety (Miles *et al.*, 1968). According to this theory, if the torsion angle (Donohue and Trueblood, 1960) of a nucleoside is fixed, substituents on the base and the sugar moiety give a change in the direction of the transition moment of the base chromophore. The sign of the rotational strength is determined by whether the polarization angle exceeds a critical value or not. If the torsion angle changes, the anisotropic effect of the sugar moiety will affect the sign of the rotational strength.

We measured CD of a variety of purine nucleosides and their 5′-monophosphates, especially those having substituents in 8 position, in order to obtain information about the interaction of the base and the phosphate moiety. Among 8-substituted purine nucleosides, some compounds have cancerostatic activity (Bloch *et al.*, 1966) and relationship of the structure and the function is of interest. From the results of CD measurements, the following conclusions were drawn. (1) Purine nucleosides having bulky substituents in the 8 position exist in syn conformation. (2) Introduction of a phosphate group to the 5′-OH of anti-type nucleosides does not change

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CD profiles around the major absorption region. (3) The phosphate group, which is introduced to the 5′-OH of syn-type nucleosides has a large influence on the Cotton effect over a wide range of wavelength. (4) The effect of the 5′-phosphate appears at CD bands of 200–220 nm both in syn- and anti-type nucleosides.

Materials and Methods

Nucleosides. 8-Bromoadenosine (IIa) (Chart I) (Ikehara *et al.*, 1967; Ikehara and Kaneko, 1970), 8-bromoguanosine (IVa) (Holmes and Robins, 1965), 8-fluoroadenosine (III) (Ikehara and Yamada, 1968), 8-(2-hydroxypropyl-2)-adenosine (Va) (Steinmaus *et al.*, 1969), 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (8,2′-*S*-cycloadenosine) (VIIa) (Ikehara and Tada, 1967), and 8,3′-anhydro-8-mercapto-9-β-D-xylofuranosyladenine (8,3′-*S*-cycloadenosine) (VIIIa) (Ikehara and Tada, 1967) were synthesized as described in the literature. Adenosine (Ia) was obtained from Waldhof Zellfabrik A. G.

Nucleotides. 8-Bromo-AMP (IIb) (Ikehara and Uesugi, 1969), 8-bromo-GMP (IVb) (Ikehara *et al.*, 1969a), and 8,2′-*S*-cycloadenosine-5′-MP (VIb) (Ikehara and Uesugi, 1970) were synthesized as described in the literature. AMP (Ib) was obtained from Schwarz BioResearch Ltd.

8-(2-Hydroxypropyl-2)-adenosine-5′-MP (Vb) was synthesized by the procedure reported for synthesis of Va (Steinmaus *et al.*, 1969). Purification was performed on a column of Dowex X-2 (formate form) resin, which was eluted with 0.1 N formic acid: uv, λ_{max}^{pH2} 260 nm (ε 15,000), λ_{max}^{pH7} 262 nm (ε 14,600); paper electrophoresis, R_{AMP}[1] 0.90.

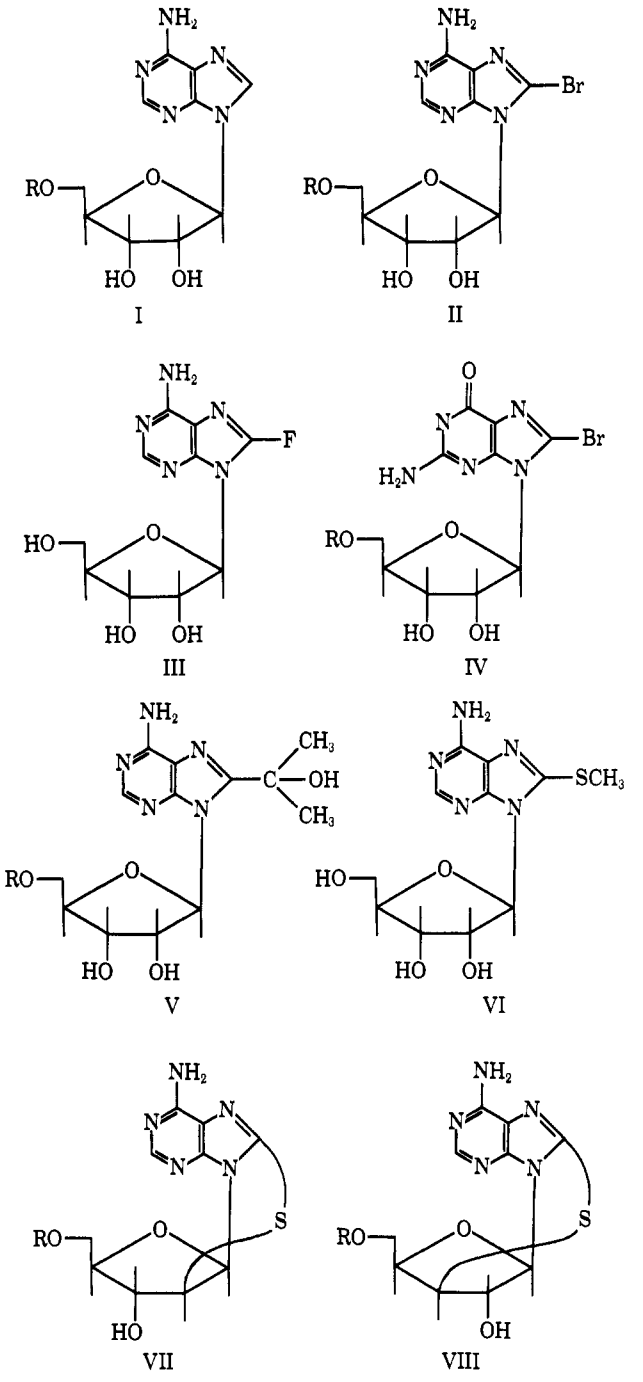
8,3′-*S*-Cycloadenosine-5′-MP (VIIIb) was synthesized from 8,3′-*S*-cycloadenosine (0.2 mmole) phosphorylated with $POCl_3$–trimethyl phosphate reagent (Yoshikawa *et al.*, 1967) (330 l.), which was prepared from $POCl_3$ (4.8 ml) and trimethyl phosphate (6 ml), at 0° for 8 hr. The reaction mixture was applied to a column of Dowex 1-X2 (formate form) and eluted with 0.2 N formic acid: uv, λ_{max}^{pH2} 282.5 nm (ε 22,300), λ_{max}^{pH7} 282 nm (ε 22,000); paper chromatography, R_{AMP} 1.08. Phosphate analyses of these mononucleotides gave satisfactory values.

Measurement of CD. CD spectra were recorded with a Jasco ORD/UV-5 spectropolarimeter equipped with a CD attachment at 15°. Materials were dissolved in a concentration to obtain OD_{max} = 1.0–2.0 in the buffer of 0.1 M phosphate (pH 7) or 0.01 N HCl. Light path was 10 mm. Solvent blanks were run before and after each sample run. Each spectrum was taken at least twice until a reproducible curve was obtained. Calibration of the magnitude of the Cotton effect was made with *d*-10-camphorsulfonic acid as a standard. Nuclear magnetic resonance (nmr) spectra were taken with a Varian HA-100 spectrometer operated at 100 MHz. Tetramethylsilane was used as an external standard. Uv absorption spectra were taken with a Hitachi EPS-3T uv spectrophotometer.

Results and Discussion

8-Substituted Purine Nucleosides. Miles *et al.* (1967) reported adenosine (Ia) to have two negative Cotton bands at 215 and 260 nm in CD. We measured adenosine and various 8-substituted purine nucleosides in order to obtain information on the effect on the Cotton curve caused by the introduction of 8 substituents, which may affect the transition moment and the torsion angle in these nucleosides.

CHART I



a, R = H; b, R = PO_3H^-

8-HALOGENOPURINE NUCLEOSIDES. As shown in Figures 1 and 3, 8-bromoadenosine (IIa) and 8-fluoroadenosine (III) gave Cotton curves of the same profile. They have a negative band at around 280 nm and two positive bands around 260 and 225 nm. Comparison of the uv absorption spectra of these nucleosides to those of adenosine suggested that the Cotton effect around 260 nm would be due to B_{1u} and B_{2u} band (Clark and Tinoco, 1965) and that of 225 nm to E_{1u} band. If we com-

[1] R_{AMP} stands for the migration distance divided by that of AMP cochromatographed side by side. Solvent systems used were (A) 1-butanol–acetic acid–water (5:2:3, v/v), (B) ethanol–1 M ammonium acetate (7:3, v/v), and (C) isopropyl alcohol–concentrated ammonia–water (7:1:2, v/v).

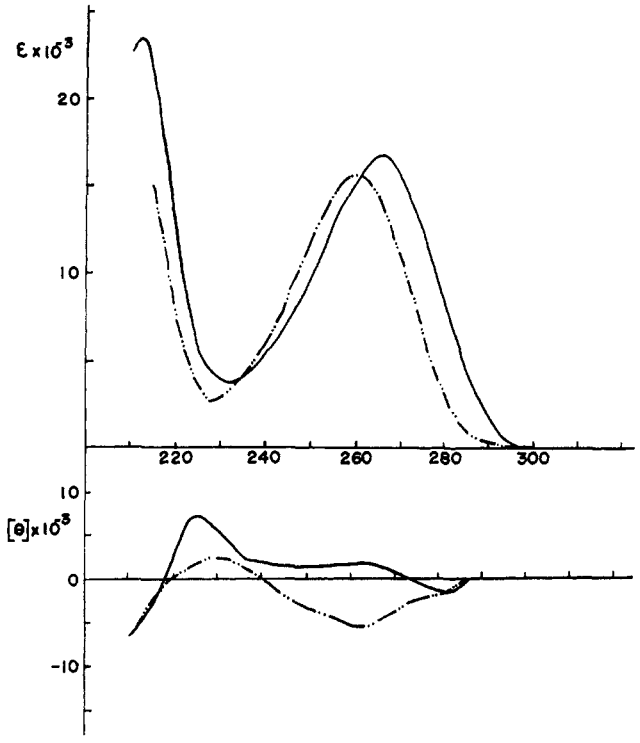


FIGURE 1: Uv absorption and CD spectra of 8-bromoadenosine and adenosine. (——) Bromoadenosine at pH 7 and (–·–·–) adenosine at pH 7.

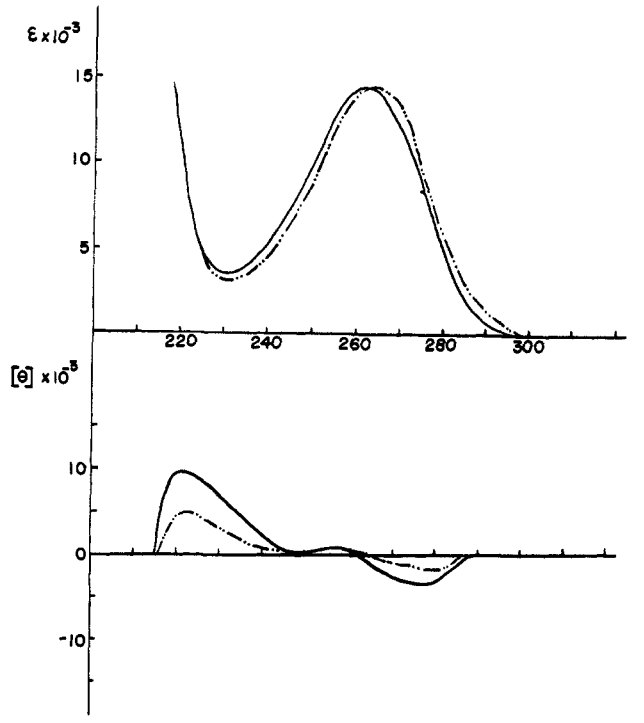


FIGURE 3: Uv absorption and CD spectra of 8-(2-hydroxypropyl-2)-adenosine and 8-fluoroadenosine. (——) 8-(2-Hydroxypropyl-2)-adenosine at pH 7 and (–·–·–) 8-fluoroadenosine at pH 7.

pare the CD curves of IIa and III to that of adenosine (Ia (Figure 1), the Cotton effect around the major absorption band is inverted in sign and that of 220–230 nm has increased in magnitude. In the curve of IIa measured in 0.01 N HCl, a maximum at 282 nm disappeared and a fairly large trough at 237 nm appeared. Therefore, the peak at 280 nm in the neutral solution could be assigned to the Cotton effect due to a $n-\pi^*$ transition. From these facts it can be said that the 8-halogen atoms in IIa and III have the same substituent effect.

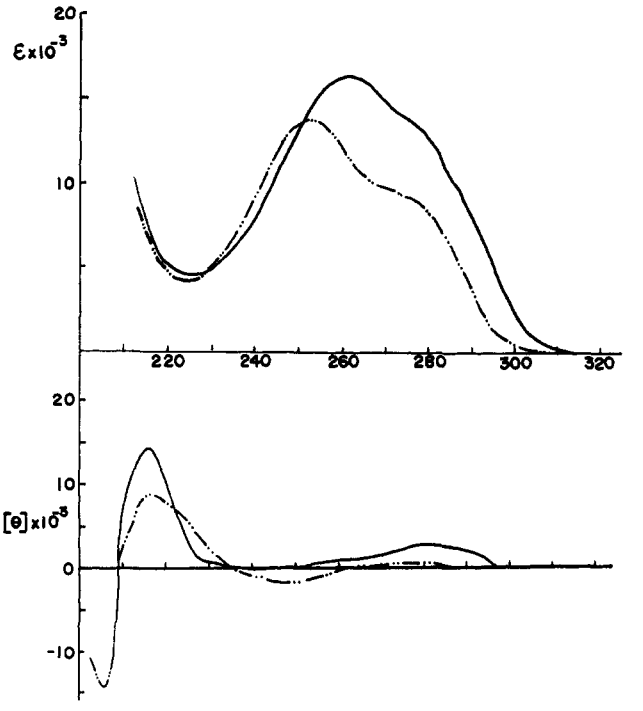


FIGURE 2: Uv absorption and CD spectra of 8-bromoguanosine and guanosine. (——) Bromoguanosine at pH 7 and (–·–·–) guanosine at pH 7.

In 8-bromoguanosine (IVa), the sign of the Cotton effect at the absorption maxima also changed to zero from the negative in guanosine (Figure 2). In the uv absorption spectrum taken in neutral solution, IVa has maxima at 263 nm and shoulder at 280 nm, whereas the maximum of CD appeared at around 280 nm as an unresolved peak. This type of degeneracy was also observed in the curve of guanosine. In the uv absorption spectrum of the latter compound, there is a maximum at 253 nm and a shoulder at 280 nm. In the CD curve the rotational strength at 253 nm is larger than observed at 280 nm.

Recently, Travale and Sobell (1970) reported that 8-bromoadenosine (IIa) and 8-bromoguanosine (IVa) are in the syn conformation in a crystalline state. Accordingly our observations that in IIa the sign of the Cotton effects due to B_{1u} and B_{2u} band were inverted with respect to those of adenosine and that the B_{1u} Cotton band in IVa was inverted with respect to that of guanosine, are well interpreted by assuming a syn conformation to these compounds. This implies that in solution 8-bromopurine nucleosides also exist in the syn conformation as in the crystal. Furthermore, the same deduction leads to the conclusion that 8-fluoroadenosine (III) also exists in the syn form. Therefore, in these three 8-halogenpurine nucleosides, a sterical interference of the 8 substituent with the furanose moiety may facilitate a stable conformation in the syn rather than in the anti form.[2]

These results are in accordance with the previous observa-

[2] From the study of polynucleotides containing 8-bromoguanosine, the same conclusion was drawn (Michelson *et al.*, 1970).

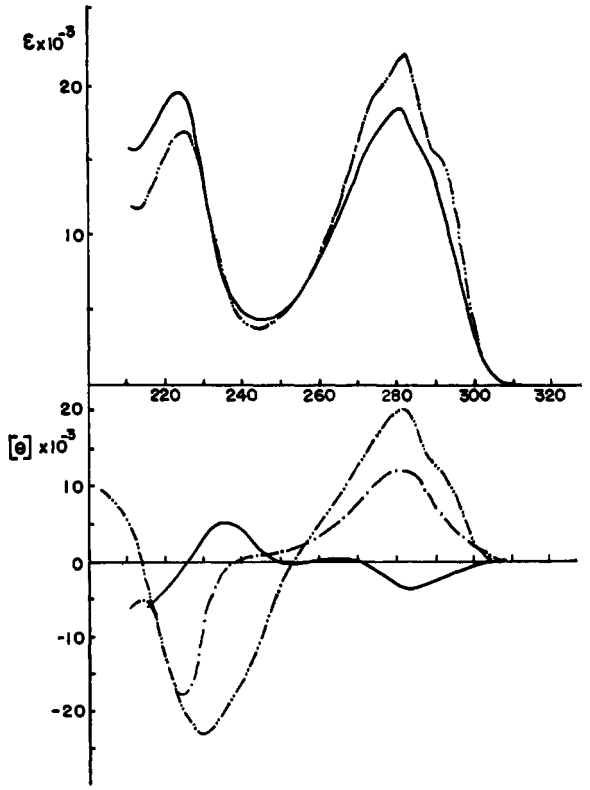


FIGURE 4: Uv absorption and CD spectra of 8-methylmercaptoadenosine, 8,2′-*S*-cycloadenosine, and 8,3′-*S*-cycloadenosine. (——) 8-Methylmercaptoadenosine at pH 7, (-·-·-) 8,2′-*S*-cycloadenosine at pH 7, and (-··-··-) 8,3′-*S*-cycloadenosine at pH 7.

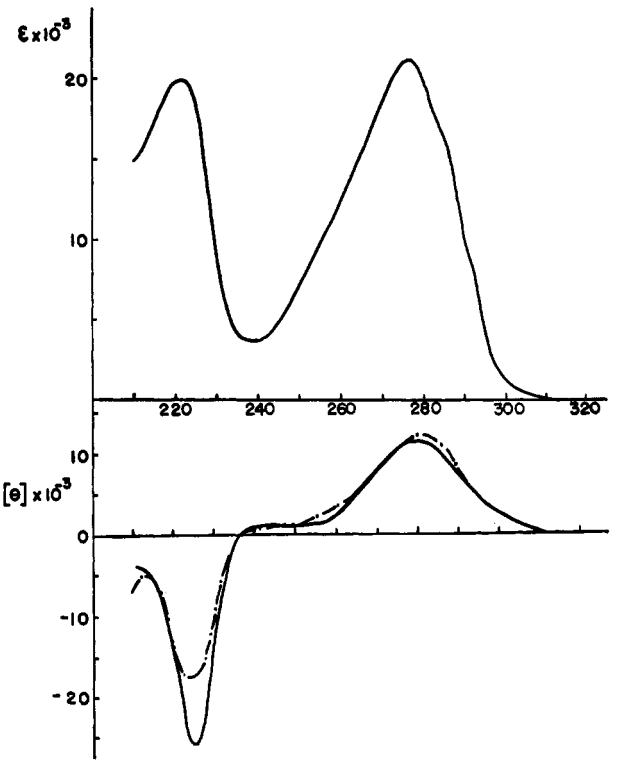


FIGURE 5: Uv absorption spectra and CD of 8,2′-S-cycloadenosine and 8,2′-*S*-cycloadenosine-5′-MP. (——) 8,2′-*S*-Cycloadenosine-5′-MP at pH 7 and (-·-·-) 8,2′-*S*-cycloadenosine at pH 7.

tion (Ikehara *et al.*, 1969b) that 8-bromo-ADP and 8-bromo-GDP are poor substrates in the polymerization reaction using polynucleotide phosphorylase. Similarly, when the copolynucleotides, poly(A,BrA) were hybridized with poly(U), BrA residues were "looped out" from the double-helical polynucleotide chain.

8-(2-HYDROXYPROPYL-2)-ADENOSINE. As shown in Figure 3, 8-(2-hydroxypropyl-2)-adenosine (Va) has a negative Cotton band at 277 nm, a small positive band at 256 nm and a large positive one at around 220 nm.

If we compare the CD of Va to that of 8-bromoadenosine (IIa), both curves have a negative band around 280 nm, a small positive around 250 nm, and a large positive around 222 nm. This may imply that the torsion angle and/or the electronic structure of these compounds are very similar. Examination of a Corey–Pauling–Koltun molecular model of 8-(2-hydroxypropyl-2)-adenosine (Va) showed that the anti form was almost impossible because of sterical hindrance between the bulky isopropyl alcohol residue at position 8 and the furanose moiety. Therefore, we concluded that Va exists in the syn form as described for 8-halogenopurine nucleosides.

8-METHYLMERCAPTOADENOSINE, 8,2′-, AND 8,3′-*S*-CYCLOADENOSINE. As shown in Figure 4, the CD spectra of 8-methylmercaptoadenosine (VIa) has a negative extreme at 283 nm and a positive one at 235 nm. In 8,3′-*S*-cycloadenosine (VIIIa), which has a uv absorption spectrum very similar to that of VIa, the sign of the Cotton bands at 280 and 230 nm is inverted and their magnitude is four to five times larger than that of the bands of VIa. 8,2′-*S*-Cycloadenosine (VIIa) also showed similar CD curves. Since in VIIa and VIIIa the base and the sugar moiety are linked by means of an *S*-anhydro linkage at ϕ_{CN} about -108 and $-70°$, respectively, the molecular conformation is rigidly fixed in any circumstances. Therefore, substituent effect at 8 position by SR group and vicinal effect by 2′- or 3′-SR group should be accumulated in the spectrum. As reported by Miles *et al.* (1968), 3′-deoxy-3′-ethylthio-2′-deoxyxylofuranosyladenine showed a relatively large negative Cotton effect, which is similar in sign to that of adenosine. Namely, 3′-ethylthio group does not exert a positive contribution to the 260-nm band. In contrast to this, in the CD spectra of VIIa and VIIIa, in which base and sugar moieties are fixed in the anti position, a positive Cotton effect appeared in the B_{2u} region. This fact may suggest that the substituent effect of the 8-SR function in the anti-type nucleoside might be one having a strong positive contribution. However, in 8-methylmercaptoadenosine (VIa) the Cotton effect due to B_{2u} transition was negative. Accordingly, the torsion angle in VIa seems to lie in the position, which exceeds 8,2′- and 8,3′-*S*-cyclonucleoside region (about -108 to $-76°$) counterclockwise and may be in the syn position. If this angle is around 180°, 8-*S*-methyl group comes to a position in which it has close contact with 2′-OH. Examination of a CPK model shows that these groups are in a distance favorable for hydrogen bonding, which may stabilize the syn conformation of VIa.

From these studies on the CD of 8-substituted purine nucleosides, it could be deduced that if a purine nucleoside has a bulky substituent at the 8 position, it exists in the syn conformation, because of steric interference between the 8 substituent and the furanose moiety.

Effect of the Phosphate Group on CD of Purine Nucleosides. Miles *et al.* (1968) reported that CD curves of adenosine and 5′-AMP are identical, because the phosphate group stretched out in water, and the distance between the phosphate and the base chromophore is too far for mutual interaction. Samejima

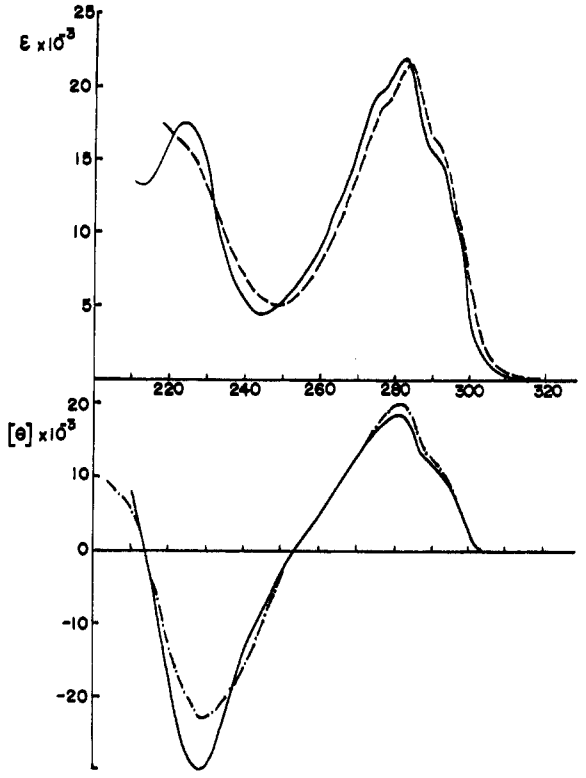


FIGURE 6: Uv absorption spectra and CD of 8,3′-*S*-cycloadenosine and 8,3′-*S*-cycloadenosine-5′-MP. (——) 8,3′-*S*-Cycloadenosine-5′-MP at pH 7, (- - - - -) 8,3′-*S*-cycloadenosine pH 12, and (-·-·-) 8,3′-*S*-cycloadenosine at pH 7.

et al. (1969) reported on CD curves of 4-thiouridine and its 2′(or 3′)-phosphates. These compounds have essentially the same CD curve in the neutral solution except that there appeared a small difference in the amplitude of the Cotton effect. We therefore measured the CD of the 5′-monophosphates of those nucleosides, which had been studied on their conformation as described above.

5′-MONOPHOSPHATES OF PURINE CYCLONUCLEOSIDES. As shown in Figures 5 and 6, if we compare CD curves of 8,2′-

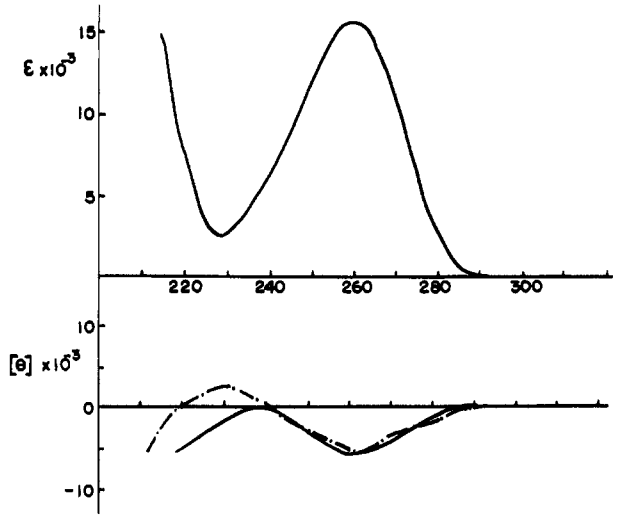


FIGURE 7: Uv absorption and CD spectra of adenosine 5′-monophosphate and aeenosine. (——) Adenosine 5-′monophosphate at pH 7 and (-·-·-) adenosine at pH 7.

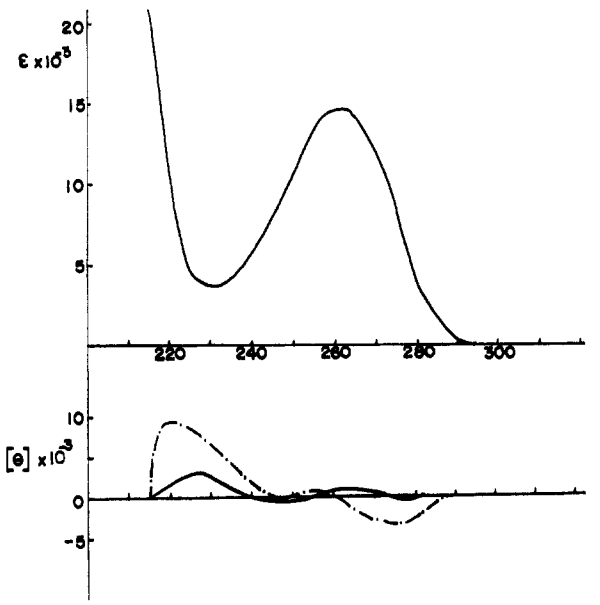


FIGURE 8: Uv absorption spectra and CD of 8-(2-hydroxypropyl-2)-AMP and 8-(2-hydroxypropyl-2)-adenosine. (—) 8-(2-Hydroxypropyl-2)-AMP at pH 7 and (-·-·-) 8-(2-hydroxypropyl-2)-adenosine at pH 7.

(VIIa) and 8,3′-*S*-cycloadenosine (VIIIa), in which the torsion angle of the base is fixed in the anti region, with those of the corresponding 5′-monophosphates (VIIb and VIIIb), almost no difference is found in the major absorption band. However, the negative Cotton effect in the 225- to 230-nm region increased significantly in magnitude by the introduction of the phosphate to 5′-OH. We, therefore, reinvestigated CD of adenosine and 5′-AMP. As shown in Figure 7, both compounds showed similar CD profiles in the long-wavelength region, but a slight difference in 220- to 240-nm region. The

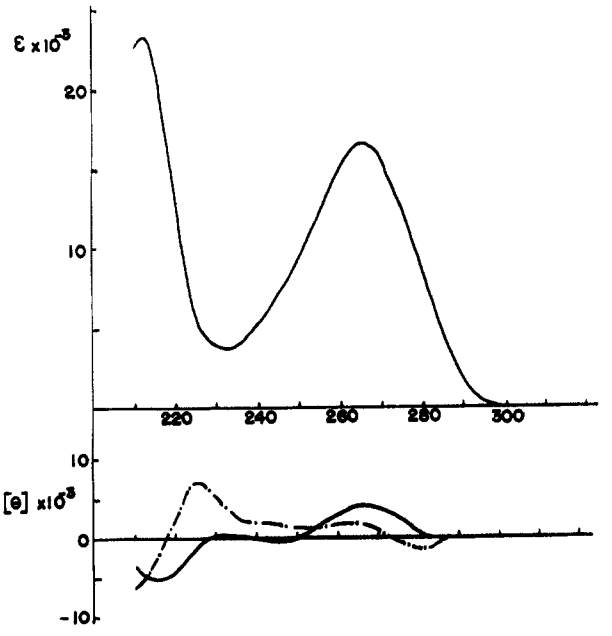


FIGURE 9: Uv spectra and CD of 8-bromo-AMP and 8-bromoadenosine. (—) 8-Bromo-AMP at pH 7 and (-·-·-) 8-bromoadenosine pH 7.

TABLE I: Nmr Signals of Purine Nucleoside 5′-Monophosphates.

	H-8	H-2	H-1′ ($J_{1'-2'}$)	H-2′	H-5′
AMP (I)	8.73[a]	8.43	6.31 (5.3)	4.97	4.25
BrAMP (II)		8.41	6.30 (5.5)	5.50	4.29
GMP	8.37		6.10 (5.6)	4.95	4.18
BrGMP (IV)			6.13 (6.0)	5.44	4.26
IspAMP (V)		8.40	6.92 (5.5)	5.52	4.31

[a] Chemical shifts were given in parts per million calculated from tetramethylsilane used as internal standard. Coupling constants were given in hertz.

conformation of adenosine (Schweizer *et al.*, 1968) and 5′-AMP (Kraut and Jensen, 1963; Schweizer *et al.*, 1968) was almost completely established as anti and those of VIa and VIIIa have to be in the anti conformation by virtue of the anhydro linkage. We can deduce, therefore, that if the base chromophore of the purine nucleoside is in the anti position, the 5′-phosphate group does not affect the rotational strength in the major absorption region, but that a significant difference appears in shorter wavelengths region.

5′-MONOPHOSPHATES OF 8-SUBSTITUTED PURINE NUCLEOSIDES. As described above, the base moiety of 8-bromo (IIa) and 8-(2-hydroxypropyl-2)-adenosine (Va) might be in the syn position. As can be seen in Figures 8 and 9, 8-bromo-AMP (IIb) and 8-(2-hydroxypropyl-2)-AMP (Vb) show a significant change in their CD curves. In the CD curve of Vb, the Cotton band at 262 nm shifted to 265 nm, and a negative band at 276 nm, as well as a positive band at 220 nm, decreased in amplitudes. Similarly in the CD curve of 8-bromo-AMP (IIb) (Figure 9), the magnitude and the sign of the Cotton effect changed significantly in a wide wavelength range. As shown in Figure 10, in 8-bromo-GMP also a change in the long-wavelength region was observed.

The syn conformation in 8-bromoadenosine (IIa) and 8-bromoguanosine (IVa) are established by a X-ray diffraction analysis. Moreover, CD curves of these nucleosides suggested the syn form as described above. The syn conformation in 8-(2-hydroxypropyl-2)-adenosine (Va) could be assumed by a molecular model building and the CD curve resembled to that of 8-bromoadenosine. Considering CD curves of 5′-monophosphates of these syn-type nucleosides, it should be emphasized that the introduction of the 5′-phosphate caused a significant change in the Cotton curve in B_{1u} and B_{2u}, as well as E_{1u} region. It could be deduced that nucleosides in which the syn conformation is preferable show a significant change in the rotational strength at a wide range of wavelengths upon introduction of 5′-phosphates. This drastic change might be interpreted as sterical interaction of the base chromophore and the phosphate. This assumption is supported by the comparison of nmr spectra of AMP (I), BrAMP (II), BrGMP (IV), and 8-isopropyl alcohol-AMP (V). As summarized in Table I, H-2′ signal of AMP (I) and GMP appeared at around 4.95–4.97 ppm. In contrast, H-2′ signals of II–V appear at 5.3–5.5 ppm. This down-field shift of H-2′ signals could be ascribed to the ring current effect and/or the magnetic anisotropy effect of N^3 atom.

From these comparable studies of purine nucleosides and their 5′-monophosphates by CD, we concluded that if a purine nucleoside has bulky group at position 8, its conformation is

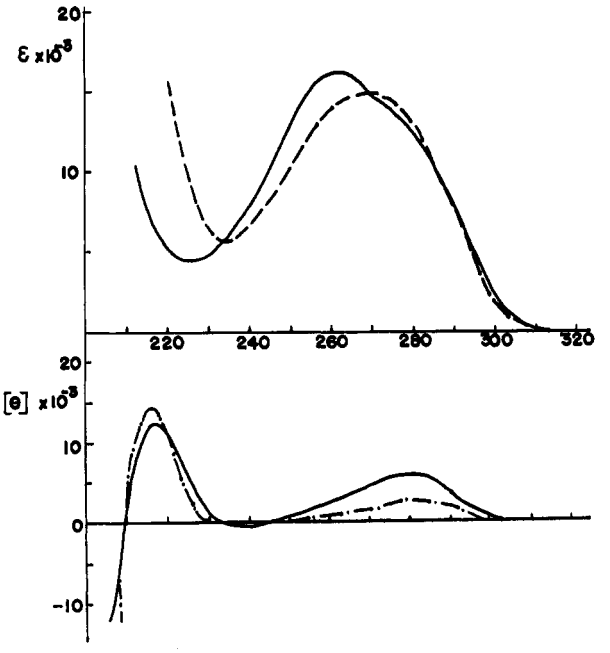


FIGURE 10: Uv spectra and CD of 8-bromo-GMP and 8-bromoguanosine. (—) 8-Bromo-GMP at pH 7, (- - - - -) 8-bromo-GMP at pH 12, and (-·-·-) 8-bromoguanosine pH 7.

syn[3] and the introduction of a phosphate group to 5′-OH causes a significant change over the whole range of the CD spectra. In contrast to this, in anti-type nucleosides, only a small change in the short wave length region is observed by the introduction of the 5′-phosphate group. Moreover, the effect of the 5′-phosphate group appear significantly in the E_{1u} band both in syn- and anti-type nucleosides and its effect appears as to increase the magnitude of the negative band and decrease that of the positive band.

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[3] After completion of this work Miles *et al.* reported on CD studies of guanine nucleosides and reached the same conclusion (Miles *et al.*, 1971).

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Conformation of Purine Nucleoside Pyrophophates as Studied by Circular Dichroism*

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ABSTRACT: The circular dichroism of dinucleoside 5′,5′-pyrophosphates of various purine nucleosides was studied for elucidation of the conformation. Pyrophosphates derived from adenosine, inosine, guanosine, and 8,3′-*S*-cycloadenosine were found to have a stacked symmetrical structure, in which bases are in anti conformation. 8,2′-*S*-Cycloadenosine 5′,5′-pyrophosphate cannot have a stacked conformation due to the restricted rotation of the bases. Pyrophosphates from 8-bromoadenosine, as well as 8-bromoguanosine, have a stacked conformation, in which bases are in syn form.

It is of importance to know the conformation of nucleic acids and their components in solution. For this purpose circular dichroism (CD) of nucleosides and nucleotides has been extensively studied (Miles *et al.*, 1969a,b; Ikehara *et al.*, 1971, 1972). Brahms *et al.* (1966, 1967, 1969) reported on the CD of oligonucleotides as models of nucleic acids. A theoretical study on optical rotatory dispersion (ORD) and CD of oligo- and polynucleotides was done by Tinoco (1964). It was shown that specific Cotton effects, which were different from those of monomers in origin, appeared by stacking of the component nucleotides. Michelson (1962) reported that P^1,P^2-dinucleoside 5′-pyrophosphates showed large hypochromicity and suggested that two bases in these molecules might be strongly stacked. In the preceding paper (Ikehara *et al.*, 1972) we have postulated conformations of various purine nucleoside 5′-monophosphates in solution as studied by CD. In this paper we describe results of the study of CD of P^1,P^2-dinucleoside 5′-pyrophosphates and discuss their conformation in solution. The pyrophosphates have symmetrical structure, and their degree of stacking is influenced by the torsion angle of the base moiety.

Materials and Methods

Synthesis of nucleoside 5′-monophosphates were described previously (Ikehara *et al.*, 1972). P^1,P^2-Dinucleoside 5′-pyrophosphates were synthesized from the appropriate 5′-monophosphate by condensation using dicyclohexylcarbodiimide (Smith *et al.*, 1961). All pyrophosphates run slower than the original monophosphate in paper electrophoresis performed in triethylammonium bicarbonate buffer (pH 7.5) and show smaller R_F values in paper chromatography in 1-butanol–acetic acid–water (5:2:3, v/v) system. Purification methods, uv absorption properties, R_F values in paper chromatography, and the mobility in paper electrophoresis are summarized in Table I.

CD was measured with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. Samples were filtered with Millipore filter and concentration of nucleotides was adjusted to 1–2 OD_{max}/ml. The measurement was performed at 15° in a 10-mm light-path cell. Calibration was made by *d*-10-camphorsulfonic acid. All runs were repeated at least twice until reproducible curves were obtained.

Uv absorption spectra were taken with a Hitachi EPS-3T spectrophotometer and phosphate analysis was made by a modified Allen's method (Allen, 1940). Solvents used were 0.001 N HCl–0.1 M phosphate buffer (pH 7.0) and 0.001 N NaOH. Molar extinction (ϵ) and molar ellipticity (θ) are presented as per residue values.

Nuclear magnetic resonance (nmr) spectra were taken with a Hitachi HA-100 spectrometer operated at 100 MHz. Samples (disodium salts) were dissolved in D_2O and lyophilized three times. Measurements were performed in D_2O solution and chemical shifts are given in parts per million relative to tetramethylsilane as the external standard.

Results

Absorption and CD Spectra of Pyrophosphates of Adenosine and Adenine S-Cyclonucleosides. For P^1,P^2-Diadenosine 5′-

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